REMARKS

Favorable reconsideration is respectfully requested in view of the following remarks.

I. CLAIM STATUS & AMENDMENTS

Kindly clarify the status of the pending claims. In item 4 on page 1 and in item 1 on page 2 of the Office Action, claims 1, 5-17 and 20-35 were incorrectly listed as pending. Instead, claims 1, 5-17 and 19-35 are the correct pending claims. Claim 19 is still pending.

Claims 14-16, 19, 21-25 and 31-34 are withdrawn.

Claims 1, 5-13, 17, 20, 26-30 and 35 were examined on the merits and stand rejected.

II. INFORMATION DISCLOSURE STATEMENT

Kindly return an Examiner-initialed PTO 1449-form indicating that the references submitted with the IDS of August 8, 2005 have been officially considered and made of record.

III. OBVIOUSNESS REJECTIONS

Claims 1, 5-13, 20, 26-30 and 35 remain rejected under 35 U.S.C. § 103(a) as obvious over Gotto et al., US 5,527,800 in view of Tobin et al., Eur. J. Pharm., vol. 281, pp. 1-8 (1995) and Lai et al., Life Sciences, vol. 62, no. 13, pp. 1179-1186 (1998). See item 2 on pages 3-4.

This rejection is respectfully traversed.

On page 2 of the Advisory Action of May 31, 2005, it is indicated that Applicant's arguments set forth in the response of May 9, 2005 are unpersuasive, because Documents 1 and 2 attached to the same response do not address the effect of Gotto's compounds 38 and 40 in Table 63 on the urinary bladder. The Examiner further notes that Gotto's compounds 38 and 40 are not distigmine, neostigmine, pyridostigmine, bethanechol and atropine, which are the compounds compared to the claimed invention in Documents 1 and 2. The Examiner concludes that the

results shown in Documents 1 and 2 are not unexpected or superior over the activity of Gotto's compounds, because Gotto's compounds 38 and 40 are the same as the two compounds recited in the instant claim 13.

In reply, kindly note that it is improper to require a comparison of the claimed invention with an invention suggested by a combination of references where the combination would necessitate a comparison of the invention with the invention itself. See M.P.E.P. § 716.02(e), last paragraph. Thus, contrary to the position in the Action, Documents 1 and 2 need not address the effect of compounds 38 and 40 in Table 63 Gotto.

Instead, Documents 1 and 2 were relied upon to compare the claimed invention with the compounds within the general class of acetylcholinesterase ("AChE") inhibitors disclosed in Gotto. Not only does the comparison show surprising and unexpected results for the claimed invention as discussed in the prior response, but it shows that there is no reasonable expectation of success that the general class of AChE inhibitors disclosed in Gotto act to improve excretory potency of the urinary bladder.

As noted on pages 4-5 of the Office Action of December 8, 2004, Gotto is relied upon as disclosing AChE inhibitors, including compounds 38 and 40 in Table 63, that fall within the formula recited in the instant claims. However, as acknowledged by the Office, Gotto fails to disclose or suggest a method for improving excretory potency of urinary bladder using such compounds. Gotto simply does not disclose the functional effects of any compounds on the excretory potency of urinary bladder.

Instead, the Office relies on Tobin and Lai as identifying acetylcholine receptors and as teaching the activation of such receptors to indirectly contribute to bladder contraction. In other words, the Office relies on Tobin and Lai as providing the suggestion to use the general class of AChE inhibitor compounds in Gotto in the claimed method for improving excretory potency of urinary bladder.

However, it is respectfully submitted that Tobin and Lai fail to provide a reasonable

expectation of success for using the AChE inhibitors disclosed in Gotto in the claimed method for improving excretory potency of urinary bladder.

As argued in the prior response, the inhibition of AChE has the possibility of leading to action, which does not result in an improvement in urination function. Consequently, there is no reasonable expectation of success that the general class of AChE inhibitors disclosed in Gotto could result in improvement in urination function.

Also, as set forth in the prior response, inhibition of AChE may contract the bladder, not only on urination, but also during the urinary storage period (collection of urine). The contraction of the bladder during urinary storage period results in decreasing the compliance of the bladder (an indicator of urinary storage potency of bladder). It also impairs the normal urinary storage function of the bladder, which causes pollakiuria and incontinence of urine.

An AChE inhibitor has the possibility of contracting not only the smooth muscle of the bladder, but also the sphincter muscle of the urethra. The contraction of sphincter muscle of urethra causes a rise in urethral resistance, and does not lead to facilitation in urination function of bladder, rather it strains excessively the bladder muscle, which results in a high pressure urination.

Moreover, Documents 1 and 2 attached to the prior response clearly show that not all AChE inhibitors function to improve excretory potency of the urinary bladder.

Specifically, Documents 1 and 2 are evidence that, the carbamate AChE inhibitors, distigmine and neostigmine, exhibited a contracting activity to the isolated detrusor muscle in the ground state (see Fig 7 and 8 of Document 1). This further decreased the compliance of the bladder in the urodynamic study using anesthetized guinea pigs.

In contrast to these carbamate AChE inhibitors, the compound of Reference Example 15 of the present invention unexpectedly showed no contracting activity at all to the isolated detrusor muscle in the ground state (see Fig 7 and 8 of Document 1). In addition, the compound did not decrease the compliance of bladder at all in the urodynamic study using anesthetized

guinea pigs (see Fig 18 of Document 2). Also, the compound of the present invention had no effect on the ability of urinary storage of the bladder.

Based on these results of the urodynamic study, it is shown that distigmine and neostigmine raised the urination pressure depending on the dosage, rather than decreasing the maximum flow rate of urine depending on the dose, and did <u>not</u> enhance the urination function. In other words, an increase in urethral resistance was observed.

On the other hand, the compound of Reference Example 15 of the present invention did not increase the urination pressure when the dosage was increased. The maximum flow rate of urine was increased depending on the dose. Thus, in contrast to the carbamate AChE inhibitor, the compound of Reference Example 15 of the present invention <u>enhanced the excretion potency of bladder without increasing urethral resistance</u> (see Fig 19 of Document 2).

Thus, Documents 1 and 2 attached to the prior response prove that not all AChE inhibitors function to improve urinary potency. Consequently, it is respectfully submitted that one of ordinary skill in the art would <u>not reasonably expect</u> that all of the AChE inhibitors disclosed in Gotto can function to improve urinary potency. Accordingly, the cited prior art references <u>lack a reasonable expectation of success</u> of using all AChE inhibitors disclosed in Gotto in the claimed method to improve urinary potency. In fact, based on the above, one of ordinary skill in the art would reasonably conclude that it would be necessary to test all of the AChE inhibitors disclosed in Gotto to ascertain whether a specific AChE inhibitor could function in the claimed method to improve urinary potency. Clearly, this does <u>not</u> amount to a reasonable expectation of success.

Furthermore, as noted on page 24 of the prior response, the compounds in the present invention not only have a potent contracting activity for the bladder muscle, but they also unexpectedly act selectively on the bladder on urination without impairing the urinary storage function of the bladder, and they do not enhance the urethral resistance, which is different from the carbonate cholinesterase inhibitors of the prior art. Consequently, the compound of the

present invention has high urination efficiency, and potent action for improving excretory potency of the urinary bladder and a therapeutic effect for dysuria not found in the prior art.

Again, although Gotto discloses compounds which are AChE inhibitors, Gotto does not disclose the action on the bladder muscle much less the effect on urinary storage function of the bladder and the action on urethral resistance. Moreover, the cited prior art references lack a reasonable expectation of success that all AChE inhibitors disclosed in Gotto function to improve urinary potency as evidenced by Documents 1 and 2 attached to the prior response. Accordingly, the cited prior art references lack a reasonable expectation of success.

In further support that the cited prior art lacks of a reasonable expectation of success and of the surprising and unexpected results of the claimed invention, attached herewith is the following reference, Hashimoto et al., The Journal of Urology, vol. 174, pp. 1137-1141 (2005). The following is a summary of this journal reference.

First, "TAK-802" in the Hashimoto reference corresponds to the compound of the present application.

The experiment in the Hashimoto reference relates to a comparison of the effects of various cholinergic drugs on voiding behavior in rats with partial urethral obstruction, which is an art recognized model for difficulty of urination. Specifically, the reference compared the selective AChE inhibitor TAK-802 (the present invention) on voiding behavior and residual urine volume in rats with partial bladder outlet obstruction with rats treated with the non-selective AChE inhibitor distigmine and the muscarinic agonist bethanechol. See the Abstract on page 1137. Features of the model are excessive residual urine, decrease of one-voiding volume and pollakiuria due to decrease of functional bladder volume, and the like. Improving effect of drugs can be estimated from the restoration of these indexes.

The results of these experiments are as follows:

(1) TAK-802 increases one-voiding volume and decreases residual urine volume without affecting voiding frequency;

(2) Although distigmine also increases one-voiding volume, the decreasing effect on residual urine volume is not dose dependent and maximum effect is less than that of TAK-802; and

(3) Bethanechol (muscarinic receptor agonist) does not affect one-voiding volume, and decreases residual urine volume by increasing voiding frequency (initiating pollakiuria). Maximum effect is less than that of TAK-802.

These results suggest that among non-carbamate AChE inhibitors, carbamate AChE inhibitors and muscarinic receptor agonists, the non-carbamate AChE inhibitors exert the most efficient effect on improving difficulty of urination. That is, distigmine, as well as TAK-802, increases one-voiding volume, but the decreasing effect on residual urine volume of distigmine is inferior to that of TAK-802. In addition, since bethanechol is an agonist, unlike AChE inhibitors and, increases constantly the tone of smooth muscle of bladder, it is considered that the voiding frequency is increased. Thus, as stated in the Abstract on page 1137, "[w]hile all 3 drugs significantly decreased residual urine volume, TAK-802 was most efficacious".

Therefore, the Hashimoto reference is further evidence that one skilled in the art would not reasonably expect that a non-carbamate AChE inhibitor is most suitable for the treatment of dysuria (difficulty of urination) based on the teachings of Tobin et al. and Lai et al.

In view of the above, the rejection of claims 1, 5-13, 20, 26-30 and 35 under 35 USC § 103(a) is untenable and should be withdrawn.

Claims 26-30 remain rejected under 35 U.S.C. § 103(a) as obvious over Gotto et al., US 5,528,800. See item 2 on pages 4-5.

This rejection is respectfully traversed for the same reasons set forth immediately above regarding the Gotto reference.

Also, as acknowledged by the Examiner, Gotto does not disclose or suggest the specific crystals of 8-[3-[1-[(3-fluorophenyl)methyl]-4-piperidinyl]-1-oxopropyl]-1,2,5,6-tetrahydro-4H-

pyrrolo[3,2,1-ij]quinolin-4-one of claims 26-30. The Examiner notes that Gotto discloses an analogous crystalline compound. However, even if the compound in Gotto inhibits AChE, this is not motivation to alter the compound in Gotto to arrive at the specific compound claimed. To assert otherwise amounts to an improper obvious-to-try rationale. Accordingly, Gotto fails to disclose or suggest each and every element of the claimed invention, and the reference lacks the requisite motivation to modify its teachings to arrive at the claimed invention.

For these reasons, the obviousness rejections are untenable and should be withdrawn.

IV. ENABLEMENT REJECTION

Claims 17 remains rejected under 35 U.S.C. § 112, first paragraph, as lacking enablement. See item 3 on pages 5-7 of the Office Action.

This rejection is respectfully traversed for essentially the same reasons set forth in the prior response and for the following reasons.

Claim 17 requires a combination therapy of an α-blocker and an non-carbamate amine compound having an AChE-inhibiting action.

The rejection is premised on the position that without guidance on what proportion of each agent to combine, mixing an α-blocker with an AChE inhibitor could be dangerous due to a potentially fatal decrease in blood pressure. In this regard, on page 7 of the Office Action, the Examiner contends that the unpredictability in the art is high, because the possible drug-drug interaction or the potentiation of adverse effects will place patients in high risk. The rejection further indicates that the state of the art does not yield any teaching for such a mixed composition.

However, as argued in the prior response, notwithstanding that the claimed pharmaceutical is effective and safe, it is well established that safety and efficacy are not to be confused with the requirements of patentability. In this regard, the M.P.E.P. at § 2107.03, V (pages 2100-45 to 2100-46) clearly indicates that "it is improper for Office personnel to request

evidence of safety in the treatment of humans, or regarding the degree of effectiveness." Similarly, the M.P.E.P. at § 2164.01(c) (pages. 2100-180) states that "[t]he applicant need not demonstrate that the invention is completely safe." Thus, it is improper to rely on safety concerns as evidence of unpredictability in the art.

Nonetheless, it is respectfully submitted that the present invention is safe and effective. For instance, regarding the safety and efficacy concerns, the mechanism of dysuria caused by prostatomegaly can be classified as: (1) a mechanical urethral obstruction caused by prostatomegaly; and (2) a functional urethral obstruction caused by hypertonia of smooth muscle of prostate. Treatment with anti-androgen and a surgical treatment to shrink the prostate are effective for the former. Therapy to release the tone in prostate and smooth muscle of urethra with a blocker, such as tamsulosin, which decreases urethral resistance is effective for the latter case.

Therefore, for prostatomegaly based on functional urethral obstruction, the combined use of α-blocker blocker and the compound of the present invention can be expected to provide a potent improving action of urination function by decreasing urethral resistance with an α-blocker, and increasing the contraction potency of the muscle of urinary bladder with an AChE inhibitor without fear of high pressure urination. Accordingly, such combination therapy is safe and useful for treating dysuria. This position was not addressed by the Office. In fact, a synergic effect was observed in the improving activity of urination efficiency as shown in Tables 8- 9 of Experimental Example 4 of the specification.

Also, regarding the diagnosis of mechanical urethral obstruction and functional urethral obstruction, the skilled clinician can easily diagnose such by ultrasound imaging, etc. Therefore the concomitant treatment of an α-blocker and a non-carbamate AChE inhibitor does not pose a risk. Thus, there is no unpredictability, because the invention can be safely and efficiently practiced.

Also, with regard to dosage and administration, the content of the non-carbamate amine

compound having AChE inhibitory action and the dosage as a therapeutic agent for urination difficulty in a combined application are described in detail on pages 108-109 of the specification.

In summary, one of skill in the art could make and safely use the claimed pharmaceutical composition comprising a combination of α-blocker and AChE inhibitor without undue experimentation given the guidance in the specification and the knowledge in the art.

Therefore, the rejection of claim 17 under 35 U.S.C. § 112, first paragraph, is untenable and should be withdrawn.

CONCLUSION

In view of the foregoing remarks, it is respectfully submitted that the present application is in condition for allowance and early notice to that effect is hereby requested.

If the Examiner has any comments or proposals for expediting prosecution, please contact the undersigned attorney at the telephone number below.

Respectfully submitted,

Yuji ISHIHARA et al.

By: Warren M. Cheek, Jr.

Warren M. Cheek, Jr.
Registration No. 33,367
Attorney for Applicants

WMC/JFW/akl
Washington, D.C. 20006-1021
Telephone (202) 721-8200
Facsimile (202) 721-8250
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EFFECTS OF THE SELECTIVE ACETYLCHOLINESTERASE INHIBITOR TAK-802 ON THE VOIDING BEHAVIOR AND BLADDER MASS INCREASE IN RATS WITH PARTIAL BLADDER OUTLET OBSTRUCTION

TADATOSHI HASHIMOTO,* HIROSHI NAGABUKURO*,† AND TAKAYUKI DOI*

From the Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited, Osaka, Japan

ABSTRACT

Purpose: We examined the effects of the selective acetylcholinesterase (AChE) inhibitor TAK-802 on voiding behavior and residual urine volume in rats with partial bladder outlet obstruction (BOO) vs rats treated with the nonselective AChE inhibitor distigmine and the muscarinic agonist bethanechol. In addition, the effect of repeat doses of TAK-802 on the bladder mass increase associated with BOO was also examined.

Materials and Methods: Male rats with BOO were used. Six to 8 days after obstruction voiding behavior was observed in a metabolic cage. The animals were then treated orally with 1 drug, and voiding frequency and urine volume at each void were measured for 3 hours. Subsequently the volume of urine retained in the bladder (residual urine) was measured. In another experiment bladder weight in rats with BOO was measured after early repeat doses of TAK-802.

Results: BOO increased voiding frequency and decreased average voided volume. TAK-802 and distigmine increased average voided volume, while not causing any change in voiding frequency. On the other hand, bethanechol increased voiding frequency without affecting average voided volume. While all 3 drugs significantly decreased residual urine volume, TAK-802 was most efficacious. In addition, bladder weight in the control BOO group was greater (approximately 2.2-fold) than that in the sham operated group and early repeat administration of TAK-802 prevented the bladder mass increase.

Conclusions: AChE inhibitors decreased residual urine volume by restoring voiding function in rats with BOO, although only the effect of TAK-802 was dose dependent. Bethanechol also decreased residual urine volume in a dose dependent manner but by increasing voiding frequency. The prevention of a bladder mass increase by TAK-802 treatment may be attributable to its effect on restoring voiding.

KEY WORDS: bladder; bladder neck obstruction; rats, Wistar; cholinesterase inhibitors; urination

Treatment for impaired bladder emptying has not been well documented despite its common occurrence. Impaired bladder emptying can be caused by chronic conditions such as bladder outlet obstruction (BOO) in men with benign prostatic hyperplasia (BPH) and impaired detrusor contractility in patients of either sex.[1,2] Clean intermittent catheterization is the most promising therapy for impaired bladder emptying, although this can cause urinary tract infections and bladder injury.[3] Pharmacotherapy using cholinomimetic drugs, such as muscarinic agonists and acetylcholinesterase (AChE) inhibitors, has been proposed as alternative therapy for impaired bladder emptying[4] since the drugs may improve detrusor contractility by activating the parasympathetic cholinergic system. However, despite experimental evidence indicating that cholinomimetic drugs increase detrusor contractility[5,6] cholinomimetic drugs have not been widely popular in clinical practice for impaired bladder emptying and only a few studies have shown beneficial effects of cholinomimetic drugs for this condition.[7,8]

Rats of either sex with BOO have frequently been used as an animal model of BPH since functional and morphological changes in the lower urinary tract associated with obstruction, that is detrusor overactivity, increased residual urine volume and bladder hypertrophy, closely resemble those seen in patients with BPH. In the current study we evaluated the effects of oral administration of the clinically used cholinomimetic drugs bethanechol, a muscarinic agonist, distigmine, an AChE inhibitor, and TAK-802, a novel selective AChE inhibitor, on voiding behavior and residual urine volume in conscious rats with BOO (fig. 1). TAK-802 is a novel AChE inhibitor with a noncarbamate chemical structure. It has been shown to have higher specificity for AChE inhibitory activity than for the inhibition of butyrylcholinesterase, which also hydrolyzes acetylcholine, and higher selectivity for muscarinic actions over nicotinic actions compared to distigmine in rats.[9] In addition, we also studied whether TAK-802 treatment can protect against the bladder mass increase caused by BOO.

MATERIALS AND METHODS

Animals. Male Wistar rats at age 11 weeks were housed in a temperature (mean ± SEM 24C ± 1C) and light (12-hour light/dark cycle) controlled room and were allowed free access to food and water. All animal experiments in this study were approved by the Takeda Experimental Animal Care and Use Committee.

Surgical procedure. The surgical procedures used to produce partial bladder outlet obstruction in rats were almost identical to those reported by Saito et al.[10] A midline longi-

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Study received Takeda Experimental Animal Care and Use Committee approval.
* Financial interest and/or other relationship with Takeda Pharmaceutical Company Limited.
† Correspondence: Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited, 2-17-85, Jusohonmachi, Yodogawa-ku, Osaka, 532-8686, Japan (telephone: +81-6-6300-6210; FAX: +81-6-6300-6306; e-mail: nagabukuro_hiroshi@takeda.co.jp).

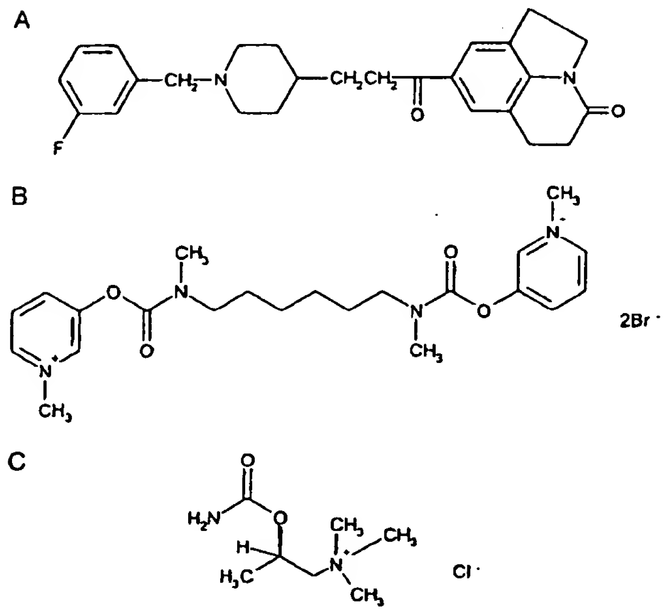

FIG. 1. Chemical structure of cholinomimetic drugs used in this study. *A*, TAK-802. *B*, distigmine bromide. *C*, bethanechol chloride.

tudinal incision was made in the suprapubic region of the abdomen with the rat under intraperitoneal sodium pentobarbital (50 mg/kg^{-1}) anesthesia. The 2 prostate lobes were retracted gently to expose the bladder neck and urethra. The urethra was tightly ligated to a glass tube 1.2 mm in diameter using a 4-zero silk suture and the glass tube was then removed. In sham operated animals only prostate retraction was performed. Each animal was housed in a separate cage until voiding function evaluation. At the beginning of each experiment animals that showed signs of urinary overflow incontinence (wetness around the external genitalia due to urine) were excluded from study.

Voiding behavior studies. Six to 8 days after the induction of obstruction voiding behavior in the animals was measured in metabolic cages that were 16 cm high, 16.5 cm wide and 20 cm deep an equipped with feces trapping mesh. Urine volume was measured using a PB1502 electronic balance (Mettler-Toledo GmbH, Greifensee, Switzerland) connected to a computer via an RS232C cable to record balance output. Balance output was fed into the computer every 30 seconds and a change in urine weight of greater than 0.05 gm was detected as a voiding episode. Following a 1-hour acclimation period after placing animals in the cages tap water was given at a volume of 10 ml/kg^{-1}. Following a 3-hour voiding measurement period after water loading (pre-drug control session) the animals received oral administration of vehicle alone or 1 test drug suspended in vehicle at a volume of 10 ml/kg^{-1}. Voided volume was then measured for another 3 hours (drug treatment session). Immediately after the completion of the drug treatment session retained urine in the bladder (residual urine) was collected by manually pressing the bladder with the animal under deep pentobarbital-Na anesthesia (100 mg/kg^{-1}). It was then measured. To confirm the severity of urethral obstruction the whole bladder was dissected out and weighed.

Repeat TAK-802 treatment effect on the bladder mass increase. One day after the induction of obstruction the animals began to be administered repeat TAK-802 doses. A 0.5% methylcellulose solution (vehicle) or TAK-802 suspended in vehicle was given orally at a volume of 10 ml/kg^{-1}. The treatment schedule was twice daily at more than 9-hour intervals for the first 3 days and once on the following day (postoperative day 4). Thus, drug was given a total of 7 times. Three hours after the last treatment retained urine in the bladder was collected from the animals under deep pentobarbital anesthesia (100 mg/kg^{-1}) and measured. The whole bladder was also dissected out and weighed. During multiple dosing with TAK-802 the animals were examined for signs of urinary overflow incontinence. Any wetting of the skin with urine was washed away with warm water and the area was dried with a paper towel.

Chemicals. TAK-802 and distigmine bromide were synthesized at Medicinal Chemistry Research Laboratories, Takeda Pharmaceutical Company Limited. Bethanechol chloride (Sigma Chemical Co., St. Louis, Missouri) was used. TAK-802 was suspended and distigmine was dissolved in a 0.5% methylcellulose solution. Bethanechol was dissolved in water.

Statistical analysis. Differences between the vehicle treated sham operated control group and the vehicle treated BOO group were compared by the 2-tailed Student's t test. Pre-drug values in every obstructed group and post-drug values in the respective control groups were compared by 1-way ANOVA. Differences in voiding parameters between the pre-drug control session and the drug treatment session were compared using the 2-tailed paired t test with Bonferroni's correction. Other differences between the vehicle treated control group and drug treated groups were compared using the 2-tailed Dunnett multiple comparison test. To compare the efficacy of cholinomimetic drugs for decreasing residual urine volume the percent of the control value for each dose of drugs was calculated as the rate of the difference between values in the sham operated vehicle treated group and values in the obstructed vehicle treated group. Drug ED_{50} values were calculated by logistic regression analysis.

RESULTS

Voiding studies. In 3 sets of experiment to examine the effects of single oral treatment of drugs no significant differences were detected in the pre-drug values of every obstructed group and or in the post-drug values in every control group. Table 1, and figures 2 and 3 show mean control group values. BOO in rats caused an increase in voiding frequency and a decrease in the average voided volume per void during the 3-hour pre-drug control session (table 1). These parameters in the sham operated group and in the vehicle treated BOO group did not change significantly during the 3-hour drug treatment session compared with values during the pre-drug session (fig. 2). TAK-802 (0.001 to 0.1 mg/kg^{-1} orally) and distigmine (0.1–1 mg/kg^{-1} orally) significantly increased average voided volume in a dose dependent manner, while not causing any change in voiding frequency (figs. 2 and 3). On the other hand, oral treatment with bethanechol (3, 10 and 30 mg/kg^{-1}) caused a significant increase in voiding frequency at every dose without affecting average voided volume (fig. 3).

Residual urine volume measured immediately after the drug treatment session in the vehicle treated BOO group was significantly increased compared with that in the vehicle treated sham operated group in our experimental setting (fig. 4). TAK-802 and bethanechol decreased residual urine volume in a dose dependent manner, while the effect of distigmine was not dose dependent and a significant effect was observed only at the dose of 0.3 mg/kg^{-1}. Of the drugs tested TAK-802 most potently and effectively decreased residual urine volume. ED_{50} for this drug was 0.0045 mg/kg^{-1} and the maximum decrease was 100% (table 2). In contrast, ED_{50} and the maximum effects of distigmine and bethanechol were much lower than those of TAK-802. The increase in bladder weight, that is an approximately 2.5-fold increase vs that in sham operated animals, was observed in each animal with

TABLE 1. *Pre-drug voiding frequency, voided volume and bladder weight in each experimental group*

Treatment	Dose (mg/kg)	No. Subjects	Mean Frequency ± SEM (No. voids/3 hrs)	Mean Voided Vol ± SEM (ml/void)	Mean Bladder Wt ± SEM (mg)
Sham operated vehicle	0.001	24	2.92 ± 0.20	1.32 ± 0.08	100.8 ± 1.9
BOO vehicle*	0.003	36	7.67 ± 0.46	0.32 ± 0.02	240.4 ± 9.0
BOO TAK-802:		10			
	0.001		7.10 ± 1.00	0.30 ± 0.04	257.5 ± 16.8
	0.003		7.20 ± 0.87	0.26 ± 0.03	240.2 ± 14.4
	0.01		7.00 ± 1.05	0.36 ± 0.04	246.0 ± 21.1
	0.03		7.70 ± 0.78	0.33 ± 0.04	290.3 ± 29.1
	0.1		6.90 ± 0.95	0.36 ± 0.05	265.6 ± 16.0
BOO distigmine:		10			
	0.1		7.10 ± 0.85	0.40 ± 0.06	244.9 ± 17.0
	0.3		7.20 ± 1.19	0.37 ± 0.05	241.6 ± 22.1
	1		6.90 ± 0.78	0.37 ± 0.05	257.7 ± 12.6
BOO bethanechol:		10			
	3		7.20 ± 0.81	0.32 ± 0.03	236.8 ± 23.7
	10		8.00 ± 1.24	0.31 ± 0.04	264.1 ± 13.4
	30		7.90 ± 0.62	0.28 ± 0.02	217.3 ± 14.3

* Vs sham operated vehicle treated control (Student's t test) p <0.01, no significant difference in all BOO groups for all parameters (on ANNOVA).

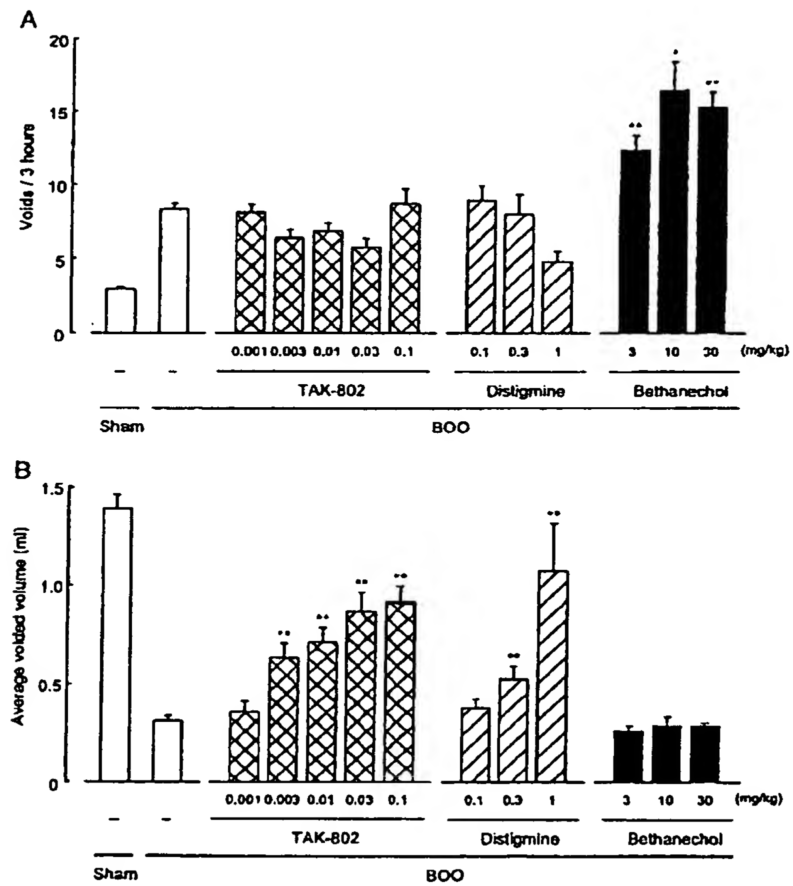

FIG. 2. Effects of TAK-802, distigmine and bethanechol on voiding behavior in rats with BOO. *A*, voiding frequency. *B*, average voided volume. Bars represent mean ± SEM in 24 sham operated animals, 36 vehicle treated animals with BOO and 10 drug treated animals with BOO at respective doses. Single asterisk indicates paired t test p <0.01 vs pre-drug controls. Double asterisks indicate paired t test p <0.01 vs pre-drug controls.

BOO. There were no significant differences in bladder weight among the BOO groups (table 1).

Repeat TAK-802 treatment effect on the bladder mass increase. Compared with that in sham operated animals 4 days of BOO led to an approximately 3.5-fold increase in residual urine volume in the vehicle treated BOO group. Repeat administration of TAK-802 (0.01 to 0.1 mg/kg^{-1} orally) decreased residual urine volume in a dose dependent manner. Bladder weight in the vehicle treated BOO group was significantly higher (an approximately 2.2-fold increase) than that in the sham operated group. Consistent with its effect of decreasing residual urine volume, repeat TAK-802 treatment suppressed the increase in bladder weight caused by BOO.

DISCUSSION

Consistent with a previous report,[10] BOO in rats in the current study caused an increase in voiding frequency and

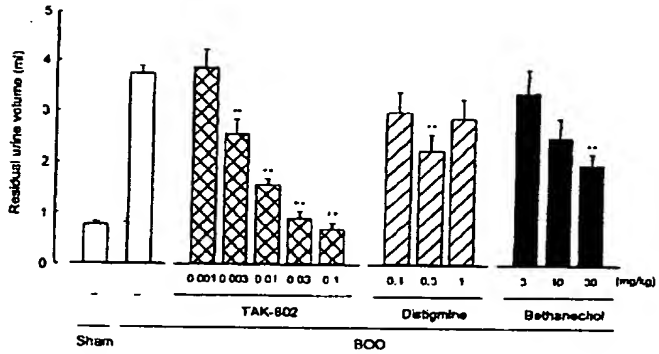

FIG. 3. Effects of TAK-802, distigmine and bethanechol on residual urine volume in rats with BOO. Three hours after drug treatment residual volume was determined in animals under deep pentobarbital-Na anesthesia. Bars represent mean ± SEM in 24 sham operated animals, 36 vehicle treated animals with BOO and 10 drug treated animals with BOO at respective doses. Asterisks indicate Dunnett's test p <0.01 vs vehicle treated BOO group.

residual urine volume, and a decrease in average voided volume. Oral treatment with TAK-802 and distigmine significantly increased average voided volume and decreased residual urine volume without affecting voiding frequency. On the other hand, bethanechol treatment caused a significant increase in voiding frequency and decrease in residual urine volume without affecting average voided volume. These differences in the effects on voiding behavior are probably attributable to the pharmacological properties of the respective drugs.

AChE inhibitors suppress hydrolysis of acetylcholine released from the parasympathetic nerve terminals and increase detrusor contractility only during the voiding phase.[11] On the other hand, muscarinic agonists cause an increase in detrusor tone and stimulate the contraction of this smooth muscle irrespectively in the filling or voiding phase. In fact, TAK-802 and distigmine have been reported to increase isovolumetric bladder contractions induced by the micturition reflex, while bethanechol has been reported to increase baseline intravesical pressure without increasing bladder contractions induced by the micturition reflex.[8] Thus, TAK-802 and distigmine increase the detrusor contraction induced by the micturition reflex, resulting in increased average voided volume, while bethanechol increases baseline detrusor muscle tone, resulting in increased voiding frequency.

Hirotsu et al reported that bethanechol improved overflow incontinence in rats with bilateral pelvic nerve transection by restoring voiding.[12] Taken together with the results of the current study it may be suggested that candidates for treatment with muscarinic agonists or AChE inhibitors are divided into 2 groups, namely subjects with loss of the micturition reflex (detrusor areflexia) and subjects in whom the micturition reflex is preserved (hypocontractile bladder). The former should be treated with muscarinic receptor agonists to induce bladder contractions to facilitate micturition and the latter should be treated with AChE inhibitors to facilitate cholinergic transmission and increase detrusor contractility during the voiding phase.

TAK-802 decreased residual urine volume in a dose depen-

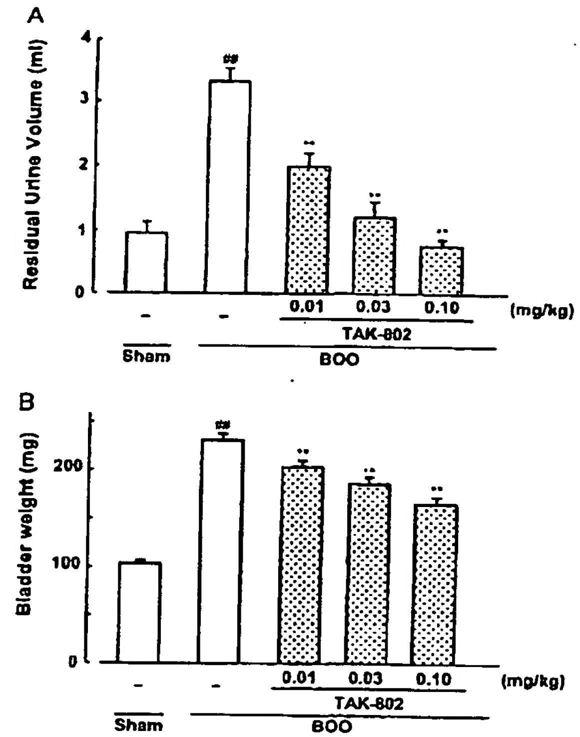

FIG. 4. Effects of early repeat oral administration of TAK-802. Vehicle or TAK-802 suspended in vehicle was given orally twice daily for initial 3 days and once on postoperative day 4, that is 7 times. *A*, residual urine volume. *B*, bladder weight. Bars represent mean ± SEM in 6 sham operated animals, 14 vehicle treated obstructed animals and 9 or 10 drug treated animals with BOO at respective doses. Pound signs indicate Student's t test p <0.01 vs sham operated control group. Asterisks indicate Dunnett's test p <0.01 vs control group with BOO.

dent manner, while the effect of distigmine on residual urine volume was not dose dependent. Our previous studies in guinea pigs have demonstrated that TAK-802 had no effect on urethral resistance and it increased the maximum flow rate of urine, while distigmine did not increase the maximum flow rate, possibly because of the increased urethral resistance caused by the stimulatory effect of the drug on the external urethral sphincter muscle.[13] Since distigmine has relatively higher selectivity for nicotinic actions over muscarinic actions than TAK-802,[8] it is speculated that distigmine might increase urethral resistance and then induce urinary retention in the current rat model of BOO. In fact, the voiding frequency tended to decrease after the administration of higher doses of distigmine.

Overactive bladder is often associated with impaired bladder emptying, which can be neurogenic or nonneurogenic in origin.[14,15] Therefore, drugs used for impaired bladder emptying should not impair bladder storage function or worsen overactive bladder symptoms. However, neostigmine and distigmine, which are carbamate AChE inhibitors, were reported to impair bladder storage function in human sub

TABLE 2. *ED_{50} and maximum effects of 3 cholinomimetic drugs on decrease in residual urine volume in BOO animals*

Drug	ED_{50} (mg/kg)	(95% CI)	Mean Max % Decrease ± SEM	Dose (mg/kg)
TAK-802	0.0046	(0.0032–0.0063)	100.0 ± 4.0	0.1
Distigmine	Not calculated		49.1 ± 9.7	0.3
Bethanechol	20.1	(not calculated)	57.7 ± 8.0	30

Ten drug treated BOO animals per group.

jects,[16] while edrophonium, a classic AChE inhibitor, was shown to induce detrusor overactivity.[17] We have also observed a decrease in bladder compliance induced by carbamate AChE inhibitors in the guinea pig[13] and acute bladder contraction was induced by intravenous administration of edrophonium (unpublished data). Thus, cholinomimetic drugs can affect bladder storage function, which may be a reason why they are not popular for the treatment of voiding dysfunction. In the current study TAK-802 showed no effect on voiding frequency. In addition, this drug has been reported not to influence bladder compliance in the guinea pig.[13] Further animal studies to investigate the effects of classic AChE inhibitors and TAK-802 on bladder storage function, including nonvoiding contractions associated with BOO, are under way.

Bladder mass has been known to rapidly increase after obstruction to overcome urethral resistance.[10] This increase in bladder weight is characterized by bladder smooth muscle hypertrophy, and hyperplasia of the urothelium and fibroblasts in the bladder with the bladder in this condition being referred to as in the compensation phase.[10,18] In the current model of BOO an approximately 2.5-fold increase in bladder mass was observed 6 to 8 days after the induction of obstruction. This increase is less than that reported previously[10] because of the shorter period after obstruction. Although a single oral treatment with cholinomimetic drugs did not change bladder weight, repeat oral administration of TAK-802 for 4 days after obstruction suppressed the increase in bladder mass induced by BOO. We speculate that TAK-802 increased voided volume and decreased the increase in residual urine volume, resulting in less bladder compensation. Thus, repeat TAK-802 administration prevented the increase in bladder mass. In a rabbit model of BOO intermittent catheterization was reported to have a similar effect on the increase in bladder mass associated with obstruction.[19] Therefore, a decrease in residual urine volume may be effective for suppressing the bladder mass increase associated with obstruction, in addition to preventing acute urinary retention.

CONCLUSIONS

TAK-802 and distigmine, which are AChE inhibitors, decreased residual urine volume by restoring voiding function in rats with BOO, although only the effect of TAK-802 was dose dependent. Bethanechol also decreased residual urine volume in a dose dependent manner but by increasing voiding frequency. The prevention of a bladder mass increase by TAK-802 treatment could be attributable to its effect on decreasing residual urine volume. The efficacy of cholinomimetic drugs has not been widely applied for impaired bladder emptying, although AChE inhibitors with a chemical structure different from that of carbamates may prove to be efficacious as pharmacotherapy for impaired bladder emptying. The clinical relevance of the findings in this study remains to be evaluated in further clinical studies.

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